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Emotional Reactivity to Incentive Downshift as a Correlated Response to Selection of High and Low Alcohol Preferring Mice and an Influencing Factor on Ethanol Intake

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Abstract

Losing a job or significant other are examples of incentive loss that result in negative emotional reactions. The occurrence of negative life events is associated with increased drinking (Keyes et al., 2011). Further, certain genotypes are more likely drink alcohol in response to stressful negative life events (Blomeyer et al., 2008; Covault et al., 2007). Shared genetic factors may contribute to alcohol drinking and emotional reactivity, but this relationship is not currently well understood. We used an incentive downshift paradigm to address whether emotional reactivity is elevated in mice predisposed to drink alcohol. We also investigated if ethanol drinking is influenced in High Alcohol Preferring mice that had been exposed to an incentive downshift. Incentive downshift procedures have been widely utilized to model emotional reactivity, and involve shifting a high reward group to a low reward and comparing the shifted group to a consistently rewarded control group. Here, we show that replicate lines of selectively bred High Alcohol Preferring mice exhibited larger successive negative contrast effects than their corresponding replicate Low Alcohol Preferring lines, providing strong evidence for a genetic association between alcohol drinking and susceptibility to the emotional effects of negative contrast. These mice can be used to study the shared neurological and genetic underpinnings of emotional reactivity and alcohol preference. Unexpectedly, an incentive downshift suppressed ethanol drinking immediately following an incentive downshift. This could be due to a specific effect of negative contrast on ethanol consumption, or a suppressive effect on consummatory behavior in general. These data suggest that alcohol intake either doesn't provide the anticipated negative reinforcement, or that a single test was insufficient for animals to learn to drink following incentive downshift. However, that high drinking and emotional intensity following incentive downshift provide initial evidence that this type of emotional reactivity may be a predisposing factor in alcoholism.

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Introduction

The occurrence of negative life events is associated with problematic drinking (Keyes, Hatzenbuehler et al. 2011). Alcohol consumption to alleviate a negative emotional state has also been consistently cited as a drinking motive (DeMartini and Carey 2011, Adams, Kaiser et al. 2012). Other recent studies have related certain genotypes with increased alcohol consumption in the face of stressful negative life events (Covault, Tennen et al. 2007, Blomeyer, Treutlein et al. 2008). Predisposition for emotional reactivity may be associated with a propensity to drink alcohol, though in human studies, it is often unclear if emotional reactivity precedes or follows problematic drinking.

Successive negative contrast, reward downshift, or incentive downshift procedures have been widely used to model emotional reactivity in rodents (Crespi 1942, Flaherty 1996). During pre-shift sessions, controls have access to a low magnitude reward and shifted animals have access to a high magnitude reward. During post-shift sessions, all of the animals have access to the low reward: that is, the reward magnitude is decreased in the shifted, but not in the unshifted group. Responding or consumption in the shifted group below the level of the control group is called a negative contrast effect, and is driven by the relative change in reward magnitude, rather than its current absolute value. The behavioral, pharmacological, and neuroanatomical data on incentive downshift suggest that contrast behavior is affectively motivated (Flaherty 1996, Papini, Wood et al. 2006). Contrast effects have also been demonstrated using human lab tasks, making successive negative contrast (SNC) a translatable procedure (Specht and Twining 1999, Anderson, Munafo et al. 2012).

High Alcohol Preferring (HAP) and Low Alcohol Preferring (LAP) mice were bi-directionally selected during 4 weeks of free-choice ethanol access, with the highest HAP intakes exceeding 20 g/kg/day (Grahame, Li et al. 1999, Oberlin, Best et al. 2011). All HAP lines drink above the rate of their alcohol metabolism and reach pharmacologically relevant blood ethanol concentrations during free-choice access, thus constituting a relevant rodent model of alcoholism (Matson and Grahame 2013). Alleles determining alcohol preference may also affect other phenotypes, providing information about mechanisms underlying differences in alcohol drinking (Flint and Mackay 2009).

Reactivity to reward downshifts is a relatively universal phenomenon that likely evolved to support foraging behavior, and may be a major source of affective reactions in humans and other species (Papini 2003). Certain individuals may react more strongly to and/or be less likely to recover from incentive downshift events. One example exists in the preclinical literature of reactivity to incentive downshift being related to an addictive phenotype. Lewis rats exemplify a addictive phenotype compared to Fisher rats (Kosten and Ambrosio 2002), and Lewis rats also demonstrate a larger and longer-lasting contrast effect compared to Fisher rats (Freet, Tesche et al. 2006). However, Lewis and Fisher rats are two inbred strains, and in order to establish a true genotypic correlation, 8 inbred strains or outbred selected lines should be compared (Crabbe, Phillips et al. 1990). An alternative strategy, pursued here, is to examine replicated selected lines. We hypothesized that reactivity to an incentive downshift would be positively correlated with selection for high alcohol preference.

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Alcohol has anxiolytic properties (Becker and Flaherty, 1983; Kliethermes et al., 1993), and may act to reduce frustration occurring as a result of an incentive downshift event. Ethanol may also inhibit a negative affective state, allowing for negative reinforcement. This idea is similar to the "tension reduction hypothesis", which maintains that individuals consume alcohol to alleviate anxiety or negative feelings (Sher 1987, Sinha 2001). Early preclinical consummatory incentive downshift data suggest that ethanol administration during recovery from contrast, after the initial reaction on post-shift day 1, attenuates contrast (Becker and Flaherty 1982, Becker and Flaherty 1983). Two additional instrumental contrast studies by Cox and colleagues (1987, 1988) suggest that ethanol reduces contrast during all post-shift days. Cox et al. (1988) showed that alcohol consumption prior to incentive downshift also prolonged recovery from incentive downshift. It is possible the different results were due to use of different downshift procedures (Flaherty 1996). Cox and colleagues (1988) also investigated activity levels immediately following contrast in animals that had consumed alcohol. Alcohol increased activity levels in both shifted and unshifted animals, but the shifted group that consumed alcohol had higher activity levels than the unshifted group that consumed alcohol, suggesting alcohol reduced the suppressive effects of contrast on locomotion. When alcohol was administered on post-shift day 2, contrast was attenuated, but it returned in shifted animals on post-shift day 3, showing that alcohol's attenuating effects are temporary. It is possible that reactivity to incentive downshift confers an increased drinking risk for individuals with a predisposition to drink because drinking transiently reduces frustration.

An additional aim was to assess whether contrast would alter subsequent ethanol consumption. The crossed HAP (cHAP) line is a cross of the HAP1 and HAP2 replicate lines, which was selectively bred with the idea that a cross of the parent lines would fix a higher number of alleles relevant for alcohol preference. The cHAP line drinks more alcohol than either parent line, achieving mean intakes in excess of 25 g/kg/day and blood ethanol concentrations (BEC) greater than 250 mg/dl (Oberlin, Best et al. 2011, Matson and Grahame 2013). Therefore, the cHAP line is an excellent genetic model of excessive alcohol consumption. In experiment 2, we measured alcohol consumption in cHAP mice immediately following an incentive shift. We hypothesized that if alcohol provides negative reinforcement, incentive downshift would increase subsequent alcohol consumption in cHAP mice.

Methods

Subjects and Apparatus

In experiment 1, subjects included HAP2 (12 m, 12 f) and LAP2 (10 m, 12 f) mice from the 46th generation, and HAP3 (12 m, 12 f) and LAP3 (12 m, 12 f) mice from the 20th generation of selection. In experiment 2a, subjects included cHAP (16 m, 16 f) and experiment 2b subjects included cHAP (12 m, 12 f) mice from the 25th generation of selection. Mice were aged 67-89 days at the beginning of training and were alcohol naive. Mice were maintained on a reverse light dark cycle (lights on 2000-0800) for at least two weeks prior to testing, and were individually housed 1 week prior to testing and throughout the experiments.

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Twelve identical operant boxes were used in all of the experiments, 21.6 × 19.7 × 12.7 cm inside, with 2 sides constructed of clear acrylic and 2 sides of aluminum (MedAssociates, St Albans, VT). Each operant box was contained in a sound and light attenuating chamber equipped with a fan for ventilation and background noise. An LED nosepoke light was used as the houselight, and was centered on the 19.7 cm side, 6.3 cm above the floor. Below it was a retractable sipper tube with a 10-ml graduated pipette readable to ± 0.05 ml that was used to measure sucrose and ethanol intakes. Lick-o-meters were used to start the 5-min testing session. During behavioral testing, Cellsorb bedding was placed under wire grid flooring and was changed bi-weekly; the operant boxes were also cleaned with 70% ethanol at this time. Boxes were wiped down to remove sucrose and droppings after each session using a wet sponge, and clean sipper tubes were used daily. Mice were run daily during the dark cycle between 1000 and 1600, using red illumination. Control of the operant boxes and collection of data was performed via the MedPC IV software and MedPC interface cards on a computer (MedAssociates, St Albans, VT). Statistical analyses were performed using SPSS (Chicago, IL).

Experiment 1

Successive Negative Contrast—Mice were food restricted to 85% ± 5% of their baseline weight (as described in the supplement). Half of the mice in each selected line were then assigned to the shifted 32% to 4% sucrose (32-4) group; the remaining mice were assigned to the unshifted 4% sucrose to 4% sucrose (4-4) group. Subjects were assigned to treatment groups counterbalanced for sex and family of origin. The day prior to testing, mice received 1 ml of their training concentration of sucrose in the home cage in order to habituate the mice to sucrose. On days 1-10 of training, mice were placed in operant boxes with the sipper tube available, which descended at the same time each mouse was placed in its assigned box. The 5-minute access period began as soon as each mouse licked once, and sipper tubes ascended following completion of the session. Mice had access to either 32% sucrose (32-4 groups) or 4% sucrose (4-4 groups). On days 11-14, all mice received the 4% sucrose solution. The sucrose solutions were made fresh for each 14-day period, refrigerated between sessions, and placed in room temperature while the mice were habituating each day to allow them to warm.

Analyses—Mixed factorial ANOVAs using the variables Sex (female or male), Line (HAP or LAP), Group (32-4 or 4-4), and Replicate (2 or 3) were used to analyze the baseline weights, pre-shift data, and post-shift data. Days was used as a within subjects variable. Intake (in ml/kg) was analyzed as a dependent variable for the pre-shift and post-shift analyses. Each replicate was tested as a separate cohort, with both LAP and HAP lines represented. As the Group × Line interactions were *a priori* comparisons of interest, the replicates were also analyzed separately using repeated mixed factorial ANOVAs performed on pre-shift and post-shift intake (ml/kg), using Days as a within-subjects variable and Line and Group as between-subjects variables. We also present the effect sizes using partial eta squared (η^2) and generalized eta squared (ηG^2), which Bakeman (2005) recommends for repeated measures designs. We also present overall means for the intake in ml and ml/kg, and licks in Table 1.

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Experiment 2

Bihourly Free-Choice Drinking—All mice had 12 hours of free-choice access to 10% ethanol and water during the dark period, and bihourly readings were performed to confirm cHAPs reached pharmacologically relevant levels (i.e. reaching alcohol intakes above the 1 g/kg/hour rate (Matson and Grahame 2013). Water and 10% ethanol were presented in 10-ml pipettes readable to ± 0.05 ml. Intakes were read every 2 hours beginning at lights off through lights on (08:00 to 20:00). All readings were taken on the home cage to minimize spillage. In the case of a leak or incorrect reading, the median value for that animal's group and sex was used, which results in <1% of the intake scores being imputed for each experiment (Matson and Grahame 2013).

Limited Access Drinking and SNC—The following day, mice received 1 hour of ethanol access (1 bottle) in the operant boxes, with 15-minute, 30-minute, and 60-minute readings taken during the access period in experiment 2a, and 15-minute, 30-minute, 60-minute and 90-minute readings taken during the access period in experiment 2b. Animals were assigned to groups by Sex, g/kg intake in the 1-hour session, and family if possible. All mice underwent the SNC task as described, but mice were not food restricted, because we used free-choice ethanol drinking in experiment 2 and wanted to ensure ethanol consumption was not for its caloric content. Immediately following post-shift day 1, all mice received 60 minutes (experiment 2a) or 90 minutes (experiment 2b) of ethanol access with readings taken at the same intervals.

Analyses—Mixed factorial ANOVAs were used to analyze both pre-shift and post-shift sucrose intake using Days as the repeated measure, and Sex (M, F), Group (4-4, 32-4) as between subjects measures. Mixed factorial ANOVAs were also used to analyze total ethanol intake across the baseline and test day, as well as the cumulative intake and ethanol intake rates across the 1- or 1.5-hour sessions using minutes as repeated measures; Group and Sex were used as between subjects measures. A within subject measure of contrast size, shift ratios, were calculated (post-shift intake/pre-shift intake) and correlated with ethanol intake on the test day.

Results

Experiment 1

Pre-shift behavior—Mauchley's test for sphericity was significant for all ANOVAs, therefore the Greenhouse-Geisser correction was used. Pre-shift day 2 data was not included in the analyses because there was a loss of the intake data for one of the replicates that day. There were 2 additional missing intake scores, and in these cases, the mean of that animal's behavior from the day before and after were used to impute a value so those animals did not have to be removed from our repeated measures analyses. During the pre-shift period, LAP 4-4 mice consumed less than LAP 32-4 mice, but the HAP 32-4 and 4-4 groups did not differ. For intake (in ml/kg), there was an interaction of Group × Line, $F(1, 76) = 56.6$, $p < .001$, which was driven by a difference between LAP 32-4 and 4-4 groups, $F(1,43) = 30.3$, $p < .001$ (Figure 1A), while HAP 32-4 and 4-4 groups did not differ, $p > .05$. HAP 4-4 mice consumed more sucrose than LAP 4-4 mice, $F(1, 44) = 53.6$, $p < .001$, while HAP 32-4 and

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LAP 32-4 did not differ in their level of intake, p > .05. Figure 1 is collapsed across pre-shift days; we present the daily data and additional analyses in the supplementary materials.

Pre-shift behavior was also analyzed separately for each replicate. For Replicate 2 mice, there was an interaction of Group × Line for pre-shift intake (in ml/kg), $F(1, 41) = 20.9$, $p < .001$ (Figure 1B). HAP2 4-4 mice consumed more sucrose than LAP2 4-4 mice, $F(1, 20) = 66.9$, $p < .001$, while there was only a trend for HAP2 32-4 mice to consume more than LAP2 32-4 mice, $F(1, 21) = 4.1$, $p = .056$. HAP2 32-4 and 4-4 mice did not differ ($p > .05$), but LAP2 32-4 drank more sucrose then LAP2 4-4 mice, $F(1, 19) = 64.0$, $p < .001$. For Replicate 3 mice, there was an interaction of Group × Line for intake (ml/kg), $F(1, 43) = 8.8$, $p < .01$ (Figure 1C). HAP3 4-4 mice also drank more sucrose than LAP3 4-4 mice, $F(1, 22) = 21.2$, $p < .001$, but HAP3 and LAP3 32-4 mice did not differ ($p > .05$). HAP2 32-4 and 4-4 mice did not differ ($p > .05$), but LAP2 32-4 consumed more sucrose than 4-4, $F(1, 22) = 5.8$, $p < .05$.

Post-shift behavior—Mauchley's test for sphericity was significant for most of the ANOVAs, and in those cases, the Greenhouse-Geisser correction was used. HAP shifted 32-4 mice experienced a larger contrast effect than LAP shifted 32-4 mice, which was supported by an interaction of Group × Line, $F(1, 76) = 4.3$, $p < .05$. HAP 32-4 shifted mice consumed less than HAP 4-4 mice, $F(1, 45) = 26.6$, $p < .001$, and LAP 32-4 mice also consumed less sucrose than LAP 4-4 mice, $F(1, 43) = 5.5$, $p < .05$, but the significance was smaller than in HAP mice (*Figure 2A, B*). The effect sizes for HAP contrast ($\eta^2 = .371$ and $\eta G^2 = .297$) were also larger than the LAP effect sizes ($\eta^2 = .114$ and $\eta G^2 = .097$). These findings indicate that both HAP and LAP lines exhibit contrast effects, but that contrast effects are larger in HAPs. HAP 4-4 mice also consumed more sucrose than LAP 4-4 mice, $F(1, 44) = 31.5$, $p < .001$; HAP 32-4 mice consumed more sucrose than LAP 32-4 mice as well, $F(1, 44) = 34.0$, $p < .001$. HAP mice drank more sucrose than LAP mice overall ($p < .001$), Replicate 3 mice drank more sucrose than Replicate 2 mice ($p < .001$), and sucrose intake changed across Days, ($p < .05$).

Post-shift intake (ml/kg) analyzed separately for each replicate indicated there was a Day × Group × Line interaction in Replicate 2 mice, $F(3, 123) = 3.3$, $p < .05$. To minimize family-wise alpha, we used a Bonferroni correction for comparisons between the groups in each line ($\alpha = .05/8 = .006$). At this level, HAP2 mice achieved contrast on post-shift days 1-3, $ts > 3.7$, $ps < .002$. LAP2 mice did not achieve contrast on any post-shift days, though there were trends on post-shift days 2 ($p = .04$) and 4 ($p = .03$). There was a Group × Line interaction for intake, $F(1, 41) = 5.9$, $p < .05$ (*Figure 3 A,C*). HAP2 32-4 mice consumed less 4% sucrose than HAP2 4-4 mice, $F(1,22) = 20.6$, $p < .001$, LAP2 32-4 mice also consumed less 4% sucrose than LAP2 4-4 mice, $F(1, 19) = 4.8$, $p < .05$. The effect sizes for contrast were larger in HAP2 mice, ($\eta^2 = .484$ and $\eta G^2 = .376$) than in LAP2 mice, ($\eta^2 = 202$ and $\eta G^2 = .141$). HAP2 32-4 mice consumed more 4% sucrose than LAP2 32-4 mice, $F(1, 21) = 48.2$, $p < .001$; the same pattern existed for the 4-4 groups, $F(1, 20) = 52.2$, $p < .001$. In Replicate 3 mice, there was no interaction of Group × Line for intake (ml/kg), $F(1, 43) = 1.1$, $p > .05$ (*Figure 3 B,D*). Although there was no interaction, it was our a priori hypothesis to examine contrast separately in each line, and therefore univariate ANOVAs were used to compare the HAP and LAP groups. HAP3 32-4 and 4-4 groups were different, $F(1, 21) =$

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9.9, $p < .01$, indicating significant contrast, with only a strong trend for contrast in LAP3 mice ($p = .06$). The effect sizes were also larger for HAP3 contrast ($\eta^2 = .321$ and $\eta G^2 = .262$) than LAP3 contrast, ($\eta^2 = .148$ and $\eta G^2 = .123$). HAP3 4-4 and 32-4 groups also consumed more 4% sucrose than their LAP3 4-4 and 32-4 mice, $F(1, 22) = 9.1$, $p < .01$ and $F(1, 21) = 11.3$, $p < .005$, respectively. We also calculated percent of control analyses for post-shift days, this analysis is presented in the Supplementary Materials. In the absence of a Group × Day interaction for replicate 3 mice, no analyses by day were conducted.

Experiment 2

Successive Negative Contrast—For experiments 2a and 2b, mixed factorial ANOVA indicated that pre-shift intake (ml/kg) increased across pre-shift days, $F(8, 224) = 5.8$, $p < .001$ and $F(4.1, 83.2) = 7.7$, $p < .001$, respectively, and the groups did not differ across pre-shift days. ANOVAs for post-shift intake (ml/kg) indicated that contrast occurred, as 32-4 mice consumed less sucrose than 4% mice on test days for experiments 2a and 2b, $F(1, 28) = 19.1$, $p < .001$ and $F(1, 20) = 11.0$, $p < .005$, respectively. The graphs of this data are presented in the supplementary materials.

Ethanol intake

Experiment 2a: All mice reached an intake rate of at least 1 g/kg/h during the first 12 hours of access, indicating mice encountered the pharmacological properties of ethanol (data not shown). There was no difference in overall ethanol intakes between the baseline and test days, but females consumed more ethanol than males, $F(1, 28) = 4.7$, $p < .05$.

The 32-4 and 4-4 groups differed in intake rates during the ethanol test session. A mixed factorial ANOVA for intake rate (g/kg/15 min) across the time-points revealed an interaction of Time × Group, $F(2, 56) = 11.0$, $p < .001$. (*Figure 4A*). Bonferroni post-hoc comparisons were performed to compare the groups at each time-point ($\alpha = .05/3 = .017$). The 32-4 mice consumed alcohol at a lower rate than 4-4 mice during first 15 minutes, but consumed alcohol at a higher rate during the last 30 minutes of the session, $ts > \pm 2.9$, $ps < .01$. The groups did not differ between15 to 30 minutes, although there was a trend for a group difference ($p = .03$). There was also a Time × Sex interaction, $F(2, 56) = 3.5$, $p < .05$. Intake rates did not change across time in either sex, $Fs < 3.2$, ps .05. Bonferroni post-hoc comparisons were performed to compare the sexes at each time-point ($\alpha = .05/3 = .017$). Females had higher intake than males at the first time-point, $t(30) = 2.8$, $p < .01$, but the sexes did not differ at the other 2 time-points.

Another mixed factorial ANOVA was performed on cumulative ethanol intake following the first post-shift day, including Sex and Group as between-subjects measures and cumulative intake per time-point as a within subjects measure. There was a Time × Group interaction, $F(1.4, 39.6) = 11.2$, $p < .005$ (*Figure 4B*). Both 4-4 and 32-4 group intakes increased across time, $F(1.4, 20.6) = 25.5$, $p < .001$ and $F(2, 30) = 100.3$, $p < .001$, respectively. Bonferroni post-hoc comparisons between the groups were performed for each of the time-point ($\alpha = .05/3 = .017$). The 4-4 group had higher intake during the first 15 minutes, $t(30) = 2.9$, $p < .01$, but the groups did not differ at 30 minutes or at 60 minutes, although there was a trend for a group difference at 60 minutes ($p = .06$). Females also consumed more than males per

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time-point, F(1, 28) = 9.0, $p < .01$. There was a significant positive Pearson's correlation of the shift ratio for intake and total ethanol intake in 32-4 mice, r = .52, $p < .05$, indicating larger contrast correlated with lower ethanol intake. There was no significant Pearson's correlation of the shift ratio for intake and ethanol intake for 4-4 mice, r = −0.06, $p > .05$ (*Figure 4C,D*).

Experiment 2b: All mice reached an intake rate of at least 1 g/kg/h during the first 12 hours of access, indicating they encountered the pharmacological properties of ethanol (data not shown). Ethanol intake was higher on the test day then on baseline, F(1, 19) = 14.2, $p < .005$.

A mixed factorial ANOVA for intake rate (g/kg/15 min) across the time-points revealed an interaction of Time × Group, F(2.3, 45.7) = 3.4, $p < .05$. (*Figure 5A*). Bonferroni post-hoc comparisons were performed to compare the groups at each time-point, resulting in an alpha level of ($\alpha = .05/4 = .0125$). Replicating Experiment 2a, the 32-4 group consumed less alcohol than the 4-4 group during first 15 minutes, t(22) = 2.8, p < .013, but in this experiment the groups did not differ at any of the other timepoints, ts < ± 1.6, *p*s > .013. Overall, 32-4 mice also consumed ethanol at a slower rate than 4-4 mice ($p < .05$). Another mixed factorial ANOVA for cumulative ethanol intake (total g/kg at each time-point) indicated there was a main effect of Group, F(1, 20) = 4.9, p < .05, with 32-4 mice consuming less overall than 4-4 mice (*Figure 5B*). There was a significant positive Pearson's correlation of the shift ratio and total ethanol intake in 32-4 mice, r = .59, $p < .05$, indicating larger contrast correlated with less ethanol intake. There was no correlation for the shift ratio and ethanol intake for 4-4 mice, r = −0.29, $p > .05$ (*Figure 5C, D*).

Discussion

Studying reward loss might be important for understanding human behaviors, because negative life events often involve an unexpected omission of a reward, such as the loss of a job. Predisposed individuals that react more strongly to reward loss or downshift may be at a greater risk for problematic drinking behavior, either because they drink to reduce frustration associated with a negative occurrence (Sher 1987), or because drinking itself may be conceived as an emotionally reactive response to stress. We present strong evidence of a genetic correlation between reactivity to incentive shift and alcohol preference, which is supported by the observation that in both replicate lines, HAPs experience larger contrast effects than LAPs. We also demonstrate that ethanol consumption is suppressed immediately following an incentive shift in HAP mice, suggesting contrast transfers from sucrose consumption to ethanol consumption.

It is our general hypothesis that emotional reactivity to incentive shifts is related to an individual's reward sensitivity or level of reward-seeking. This hypothesis is supported by the observations that HAP mice consumed more sucrose than LAP mice, confirming earlier studies of saccharin intake in these mice (Oberlin et al., 2011; Grahame et al., 1999). Therefore, HAP mice may have higher consummatory drives for rewards compared to LAP mice. Others have found associations between ethanol intake and sweet preference, in rat high and low alcohol preferring lines and inbred strain panels (Belknap, Crabbe et al. 1993,

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Phillips, Crabbe et al. 1994, Stewart, Russell et al. 1994, Kampov-Polevoy, Kasheffskaya et al. 1996). Conversely, Agabio and colleagues (2000) found no evidence for a correlated response in saccharin preference between Sardinian Alcohol Preferring and Non-preferring rats.

Line differences in sucrose consumption may be a limitation of this experiment, because one could argue that we were more sensitive to detecting contrast effects in HAPs because of their higher baseline sucrose intake. There is evidence that differences in reward sensitivity predict differences in contrast behavior. Therefore, baseline differences in pre-shift intake may be common if one is studying individual differences in contrast because animals with greater avidity may experience greater contrast and emotional reactivity when a reward is decreased in value. Freet and colleagues (2006) observed a similar pattern of pre-shift behavior in Lewis and Fisher rats, where Lewis rats had higher pre-shift sucrose intake than Fisher rats and also exhibited a larger contrast effect than Fisher rats during the post-shift period. Interestingly, selection for high and low contrast also resulted in pre-shift intake line differences (Flaherty, Krauss et al. 1994). The Large Contrast line had a higher level of licks during pre-shift compared to the Small Contrast line, an effect that was present across several generations of selection. Conversely, no pre-shift difference existed for Maudsley Reactive and Maudsley Non-Reactive rats, even though Maudsley Non-Reactive rats exhibited a larger contrast effect (Rowan and Flaherty 1991). Therefore, it is not always the case that individual differences in contrast occur in conjunction with individual differences in pre-shift intake. Although LAP mice had a low level of 4% sucrose intake, we reasoned that contrast was possible because intake of 4% sucrose was statistically different from zero. Further, though contrast may be affected by differences in avidity for sucrose, the occurrence of contrast completely relies on between-group contrasts conducted within-line, and may therefore not be as affected by line differences in baseline intake.

Experiment 1 demonstrates that HAP mice experience larger contrast effects than LAP mice. This assertion is supported by the interaction of Line × Group, as well as larger effect sizes for contrast in HAP lines than the LAP lines. This observation is particularly important because effect sizes describe magnitude, thus providing descriptions of stronger contrast in HAP mice than in LAP mice. Considering the follow-up comparisons and effect sizes for contrast in each Line and Replicate, there is moderate to strong evidence for a genetic correlation between alcohol preference and emotional reactivity to an incentive shift. As discussed, Freet and colleagues provided initial evidence that a drug-seeking phenotype might be associated with reactivity to incentive downshift in Lewis rats compared to Fisher rats, but these results are limited because Lewis and Fisher rats are inbred strains. Because the strains are isogenic, the results cannot be inferred to be representative of the general population. Here, two sets of High and Low Alcohol Preferring lines provide higher confidence in the genetic correlation than a single comparison between two inbred strains (Crabbe et al., 1990).

Overall, it seems that when HAPs expect a reward will be available, they readily consume it. If the reward is reduced unexpectedly, HAP consummatory behavior is suppressed. Here, we unexpectedly showed that this suppression of consummatory behavior transfers to ethanol consumption. Because our design ensured all animals had previously encountered the

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pharmacologic, and presumably pleasant effects of ethanol prior to the downshift, we hypothesized mice would draw upon this experience and drink to counteract the frustration associated with the downshift. Because we chose this design, we also chose not to include LAP mice in the experiment because they drink insignificant quantities of alcohol. Instead, ethanol intake was suppressed immediately following the incentive downshift (*Figures 4, 5*). In both experiments 2a and 2b, on post-shift day 1 ethanol intake in the shifted mice was immediately suppressed, but increased after 15 minutes of access time above or to the level of the 4-4 mice. In experiment 2a, there was a trend for the 32-4 mice to consume more ethanol than the 4-4 mice overall, suggesting there may have been a rebound effect in drinking, but this pattern did not reappear in experiment 2b. On the other hand, there was a positive correlation between level of drinking and sucrose shift ratios in both experiments 2a and 2b, suggesting that the smaller the contrast, the larger amount of ethanol consumed (*Figure 4, 5*). Overall, these data suggest that contrast suppressed ethanol consumption. This is an unexpected result, but it is interesting because ethanol is a highly preferred substance by cHAPs. Further, HAP mice find moderate doses of ethanol to be rewarding as measured by a conditioned place preference paradigm (Grahame, Chester et al. 2001). Therefore, this study provides initial evidence that SNC reduces ethanol drinking in mice that have been selectively bred to do so. Though we did not find evidence of drinking for negative reinforcement in this study, this study highlights an interesting juxtaposition that HAP mice consume high amounts of available rewards normally, but in the face of frustration, they reduce this behavior, a statement that applies to both sucrose and ethanol. Whether mice could eventually learn to drink alcohol to attenuate the stress of reward loss remains an open question, as animals had only one opportunity to do so here. Future studies should examine if suppression of ethanol drinking is a specific effect, or if contrast decreases general consummatory behavior of other rewards following an incentive shift. In addition, as a single test may have been insufficient for animals to learn to drink following incentive downshift, a multiple test day procedure could be used.

Finding larger negative contrast effects in HAP mice compared to LAP mice provides initial evidence that emotional reactivity to incentive downshift is a potential behavioral endophenotype for alcoholism. These preclinical findings could be translated, as contrast effects have also been observed in humans (Specht and Twining 1999). We believe these findings are relevant to the way that predisposed individuals react to rewards in general; it is also possible that heightened sensitivity to reward loss could motivate drug seeking because the rewarding effects of drugs tend to be transient. Once they are absent, those most sensitive to this loss of reward may experience more intense emotional responses, resulting in greater subsequent drug seeking behavior. Future work in humans would be an important step in determining the degree to which emotional reactivity contributes to addictive behaviors. Further, learning about predisposing factors for alcoholism is important for developing preventative and tailored treatments for both aberrant emotional reactivity and the disorder. For example, if emotional reactivity associates with drinking, one could target reactivity in humans with cognitive behavioral therapy (Olatunji, Cisler et al. 2010) or pharmacological treatment (Siepmann, Heine et al. 2007) to prevent reactivity in predisposed populations or to reduce drinking. Lastly, the increased emotional reactivity

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seen in HAP mice may allow for exploration into the shared genetic and neurological mechanisms underlying emotional reactivity and high alcohol preference.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Lines of High and Low Alcohol Preferring mice were tested for emotional reactivity
- Emotionality, measured in successive negative contrast, was greater in HAP mice
- These data suggest emotionality correlates with alcohol consumption
- Findings did not support the idea that mice drink alcohol to alleviate emotionality

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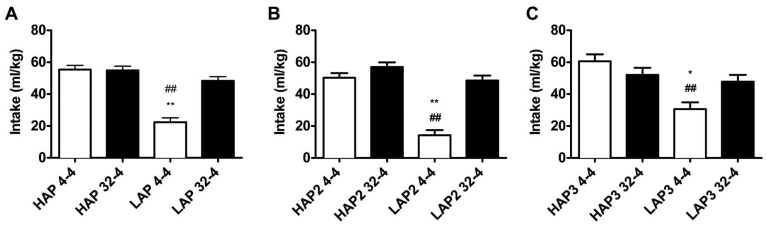

Figure 1.

Pre-shift intake in each Line (HAP, LAP), Replicate (2, 3) or Group (32-4, 4-4). (A) Overall intake during pre-shift days collapsed across replicates (B) Overall intake during pre-shift days in Replicate 2 mice (C) Overall intake during pre-shift days in Replicate 3 mice.

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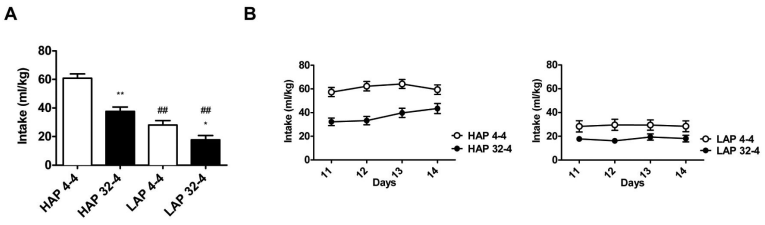

Figure 2.

Post-shift intake (in ml/kg) for each Line (HAP, LAP) and Group (32-4, 4-4). (A) Overall intake (in ml/kg) during post-shift days collapsed across replicate (B) Intake (in ml/kg) across post-shift days collapsed across replicate

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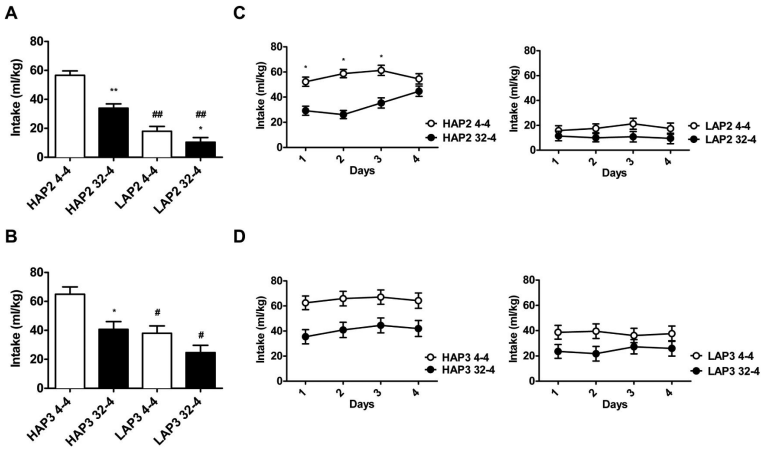

Figure 3.

Post-shift intake in each Line (HAP or LAP), Replicate (2 or 3) or Group (32-4 or 4-4). (A) Overall intake during post-shift days in Replicate 2 mice (B) Overall intake during post-shift days in Replicate 3 mice. (C) Intake in HAP2 and LAP2 mice across post-shift days (D) Intake in HAP3 and LAP3 mice across post-shift days.

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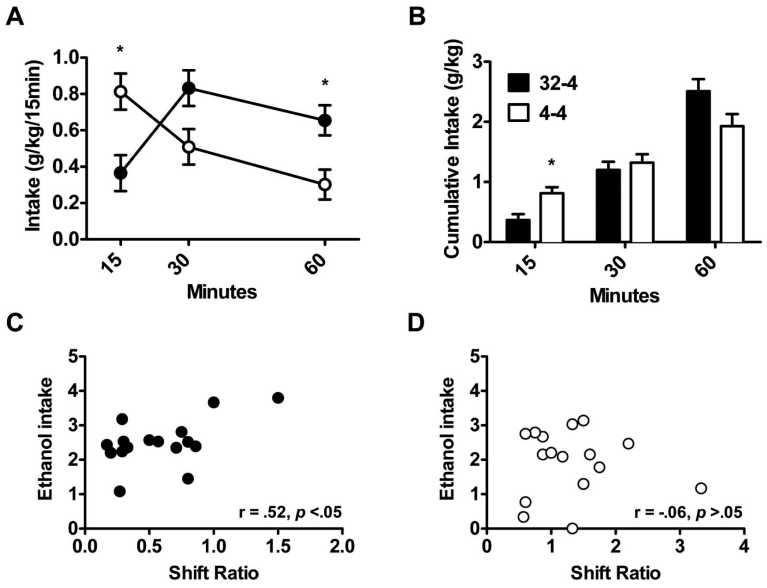

Figure 4.

Ethanol intake during a 1-hour test session following incentive downshift in cHAP mice (A) Rate of intake (g/kg/15 min) (B) Cumulative intake (g/kg) (C) Correlation between the shift ratio and ethanol intake in shifted mice (D) Correlation between the shift ratio and ethanol intake in unshifted mice

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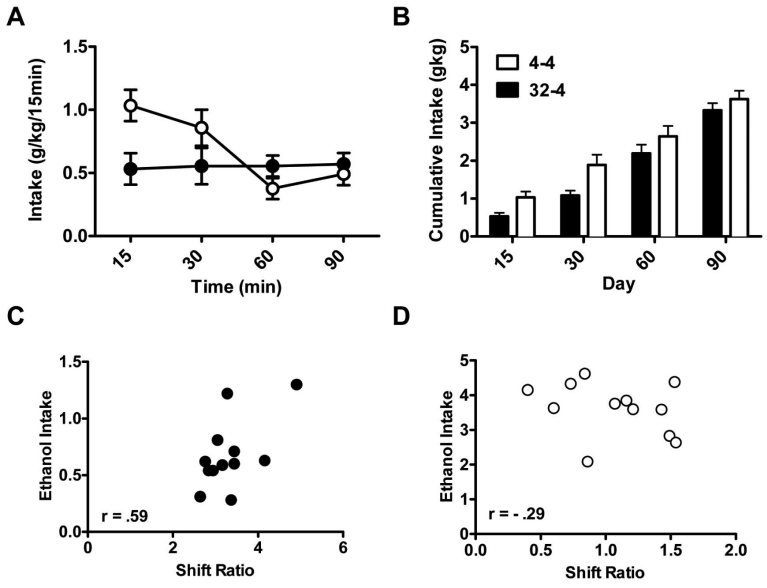

Figure 5.

Ethanol intake following a 1.5 hour test session following incentive downshift in cHAP mice. (A) Rate of intake (g/kg/15 min) (B) Cumulative intake (g/kg) (C) Correlation between the shift ratio and ethanol intake in shifted mice (D) Correlation between the shift ratio and ethanol intake in unshifted mice

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Table 1

Line and Replicate Average Weights and Consummatory Behavior

	HAP2	LAP2	HAP3	LAP3
Baseline Weight (g)	26.1 ± 0.4	24.4 ± .5$^{\#}$	24.1 ± .4	25.7 ± .4
Pre-shift				
Intake (ml) Intake (ml/kg) Licks	1.22 + .06	.66 + .06$^{\#\#}$	1.17 + .06	.85 + .06$^{\#}$
	53.71 + 2.57	31.54 + 2.76	56.71 + 2.63	39.18 + 2.57
	714.3 + 48.5	450.6 + 52.0	650.7 + 49.7	573.0 + 48.5
Post-shift				
Intake (ml) Intake (ml/kg) Licks	1.02 ± .06	.30 ± .07$^{\#\#}$	1.06 + .06	.67 + .06$^{\#++}$
	45.27 ± 3.00	14.28 + 3.22	53.17 + 3.08	31.33 + 3.00
	604.8 ± 36.5	227.6 + 39.1$^{\#\#}$	563.4 + 37.4	425.0 + 36.5$^{\#+}$

For all figures and tables, Asterisks (*) denote Group (32-4, 4-4) differences. * is significance at .05 level or lower/** is significance at .001 level or lower. Hashtags (#) indicate a Line (HAP, LAP) difference.

Plus signs (+) denote Replicate (Line 2, 3) differences.

Dollar signs ($) indicate a Sex (M, F) difference. $ is at the .05 level or lower, $$ is at the .001 level or lower.

$^{\#}$is significance at .05 level or lower,

$^{\#\#}$is significance at .001 level or lower.

$^{+}$is significance at .05 level or lower,

$^{++}$is significance at .001 level or lower.